Methods: This study is a multi-center, placebo-controlled, Phase II randomized discontinuation study in patients with advanced refractory progressive solid tumors which included advanced sarcoma patients. Initially, all patients received oral BAY at 400 mg twice-daily for12-weeks. At the end of this 12-week induction phase, antitumor responses were assessed. Patients whose target lesion tumor burden showed growth greater than 25%(progressive disease, PD) during the induction phase were discontinued from the study. Patients whose tumor demonstrated target lesion tumor burden shrinkage greater than 25% (responders) were not randomized and continued BAY in an open label phase, until disease progression or toxicity. Patients with tumor target lesion measurements that remained within 25% of the baseline pretreatment measurements (stable disease, SD) were randomized to receive either BAY, 400 mg every 12 hours, or matching placebo.
Results: To date 27 advanced sarcoma patients with different histologies have been enrolled of which 23 pts were evaluable for response. The median age was 56 years (range of 24y to 79y), ECOG performance status 0 (38%) and 1 (63%) and all (100%) had at least one prior systemic therapy. Seven pts discontinued study drug earlier than the 12-week assessment and 16 pts have been treated with BAY up to the 12-week assessment point. Investigator's assessment of response at the 12 week assessment point demonstrated 3 responders (continued on BAY), 5 SD (randomized to either BAY versus placebo) and 8 PD (off study) at the 12 week assessment point. Responder's histologies were 2 gastrointestinal stromal tumors (refractory to imatinib mesylate) and 1 synovial sarcoma. The most frequent drug-related toxicities included hand-foot skin reaction, rash/desquamation, anorexia, diarrhea, hypertension and fatigue.
Conclusion: While the study is still ongoing and the randomized portion of the study is yet to be analyzed, these preliminary data suggest that BAY may have potential anti-tumor activity in advanced sarcoma. Further clinical study in this setting is warranted.

383 POSTER

Preclinical antitumor activity of second generation analogs of SDX-101

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Introduction: SDX-101 is an anti-neoplastic drug with a novel mechanism of action currently in Phase II clinical trials in leukemia. SDX-101 exerts its anti-neoplastic activity by inhibiting the activity of the beta-catenin pathway, via its interaction with the PPAR-RXR/beta-catenin nuclear complex.
Aim: The aim of this project was to synthesize and assess the cytotoxic activity and the mechanism of action of a series of SDX-101 analogs created by structural modification at various positions on the parent molecule.
Results: The SDX-101 analogs were screened in cell-based cytotoxicity assays and functional assays for beta-catenin inhibition. Three lead compounds have been identified: compound # 2, compound #5 and compound #8. As is the case with SDX-101, these compounds displayed selective cytotoxic activity for malignant cells when compared to the normal cells. The IC50 observed in LNCaP prostate cancer cell line ranged from 13±3 μM (#5) to 39±13 μM (#2). Compound #5 displayed an IC50 of 48±1 μM and 28±3 μM in the prostate cancer cell lines DU-145 and PC-3 and an IC50 of 16±2 μM and 8±3 μM in the colon cancer cell lines SW-480 and HCT-116. The IC50 values obtained with each of the analogs in these prostate cancer cells were markedly lower than those of SDX-101 (range 122–505 μM). Beta-catenin inhibitory activity of these leads was confirmed by reporter-promoter assays as well as by measuring mRNA and protein levels of beta-catenin-regulated genes such as cyclin D1 in cancer cell lines. The analogs were also potent in inhibiting tumor growth of Daudi xenografts in SCID mice. Following treatment with 125–250 mg/kg/d oral dose for four weeks, the mean tumor volumes for vehicle, #5, #8, and #2, were 1543 mm3, 946 mm3, 1078 mm3 and 825 mm3, respectively ($p<0.07$ each analog vs vehicle). The tumor volumes of SDX-101(400 mg/kd/d) and chlorambucil (2 mg/kg/d) treated mice were 1157 mm3 and 864 mm3 respectively. Time for the tumors to reach eight times (8X) the initial volume (100 mm3) was markedly delayed with all SDX-101 analogs: the controls reached 8X volume in approximately 9 days compared to approximately 21 days for the analogs. The treatment was well tolerated with no mortalities and no significant body weight loss.
Conclusions: Orally effective and well tolerated SDX-101 analogs have been identified with potent anti-neoplastic activity and similar mechanism of action.

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Sulindac sulfide modulates beta-catenin dependent expression of the metastasis-associated gene S100A4/mts1

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Background: This study was designed to identify the impact of pathway modulators on the newly identified target gene of the beta-catenin/TCF pathway, the metastasis-associated gene S100A4/mts-1/metastasin.
Material and Methods: Gene knock-out technology was coupled to cDNA array analysis of the colon carcinoma cell line HCT116 (heterozygous for D45; wt/m) and a derived wt/-knock-out strain. Target gene confirmation was pursued using additional knock-out cell strains (wt/−; −/m) and a naturally nullosomic tumor cell line NCI-H28 investigating both mRNA and protein levels. Wild type and/or D45-beta-catenin-transduced clones of knock-out strains and NCI-H28 were created to prove the impact of D45-mutation on target gene expression and migration. The beta-catenin/TCF-pathway was analyzed by gel shift and reporter assays with several target gene promoter variants. In order to analyze the dependency of S100A4 expression on the beta-catenin/TCF-pathway, the modulators LiCl, known as inhibitor of the glycogen synthase kinase 3b, and sulindac sulfide, known to target the nuclear accumulation of beta-catenin, were employed.
Results: S100A4, which is associated with the metastatic phenotype, was dramatically down-regulated in wt/-knock-out strains compared with HCT116 cells and −/m knock-out strains. S100A4 expression positively correlated with the in vitro invasive phenotype. In NCI-H28/D45 cells, S100A4 levels were increased up to 70-fold, correlating with enhanced migration behavior. S100A4 promoter activity of deletion variants was dependent on beta-catenin availability and TCF-4 binding site presence. Treatment with the pathway activator LiCl led to induction of S100A4 expression in HCT116 cells and in the knock out strains analyzed. Treatment with the pathway inhibitor sulindac, however, clearly reduced the expression levels of S100A4 in these cell lines. Moreover, sulindac-treated cells showed reduced migration behavior compared with the non-treated controls.
Conclusion: S100A4 is a target gene of the beta-catenin/TCF-pathway. Mutant D45-beta-catenin allele acts in a dominant fashion to activate S100A4 expression. Modulators of beta-catenin signaling may offer potential as antimetastatic agents by interdicting S100A4 expression.

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A phase II trial to assess the efficacy and safety of Gefitinib (IressaTM) in patients with metastatic hormone refractory prostate cancer (HRPC) who progressed on treatment with a luteinising hormone releasing hormone analogue (or post orchiectomy) plus an antiandrogen

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EGFR is overexpressed in HRPC. Gefitinib enhances antiproliferative effect of antiandrogen bicalutamide when coadministered to moderately androgen-independent prostate tumour xenografts. This is a phase II trial aimed to assess activity and safety of gefitinib in patients with metastatic HRPC who progressed to an LHRH analogue plus antiandrogen. Patients received gefitinib 250 mg daily and antiandrogen plus LHRH analogue for 2 months or until disease progression (PD). Patients with PD stopped antiandrogen therapy and continued gefitinib with LHRH analogue. Thirty-four patients have been planned for the study. Global health status, pain score and quality of life (QoL) have been assessed at baseline and every 2 months by visual analogue scale, McGill-Melzack and EORTC QLC-30 questionnaires. Patients who had no surgery underwent a prostate biopsy to study EGFR and HER2 expression. Serum HER2 and EGFR extracellular domain (ECD) were evaluated every 2 months. EGFR and HER2 ECD were assayed by ELISA method. A cut-off of 15 ng/ml was used for HER2 ECD. A reference range for HER1 was determined on 30 healthy subjects sera (45.7–71.3 ng/ml). From April 2003 to May 2004 18 patients have been enrolled. Baseline data are available for all cases, 16 cases are evaluable for safety and 11 for efficacy. The median age was 65 years (range 56–78). WHO performance status was 0 in 13 and 1 in 3 patients. Seven patients received no prior surgery. Median basal PSA was 35.9 ng/ml (8.2–463.0). Median duration of treatment with gefitinib was 98 days (5–369). A PSA levels drop (>25%) respect to baseline was observed in 2 patients, and PSA stabilization in 1 case. Median time to progression

was 63 days (33–336). The main side-effects of gefitinib were grade 1–2 skin rash, diarrhea and transaminitis. Data analyses on QoL are ongoing. Four patients underwent prostate biopsy. EGFR was overexpressed in 2 patients, cerbB2 was absent in all tissue samples. Serum HER2 ECD was assessed in 12 patients. Mean basal value was 10.1 ng/ml (6.5–14.4). After 2 months mean value was 11.7 (9.0–15.7). Serum EGFR was assessed in 14 patients. Mean basal value was 55.5 ng/ml (41.4–64.8). Mean value at 2 months was 52.8 ng/ml (47.5–58.3). Gefitinib has been associated with infrequent PSA responses and no objective response in patients with metastatic HRPC. Further evaluation of data from this study will clarify the effect on QoL and the correlation between serum EGFR and HER2 and clinical outcome.

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Effect of angio-sonography to monitor response during imatinib treatment in patients with metastatic gastrointestinal stromal tumor (GIST): a preliminary report

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GIST metastases are typically intra-abdominal and hypervascular. BR1 is a new blood pool ultrasound second-generation contrast agent, which consists of stabilized microbubbles, which allows angio-sonography through continuous real time examination during different vascular phases of contrast enhancement using low transmission power. We assessed angiosonography to monitor response during imatinib treatment (400 mg orally once daily) in patients with metastatic cKIT+ GIST. Ten consecutive patients with known advanced cKIT+ GIST were investigated with angio-sonography and CT. We also monitored the serum levels of vascular endothelial growth factor (VEGF). Angio-sonography showed an early reduction in tumor vascularization in all 10 cases. The tumor perfusion appeared reduced in the central part of the GIST metastases. With a median follow-up of 15 months (range 3–21), a reduction in tumor vascularization was continuously observed in all 10 patients, but in 2 progressive disease (PD) was documented after 12 and 21 months of imatinib treatment. CT documented tumor response according to standardized criteria in 6 patients (median time to response 4 months, range 1–9), stable disease (SD) in 2 lasting 18+ and 21+ months, and PD in 2 according to angio-sonography. Serum VEGF levels behaved in an heterogeneous manner, but an early reduction in serum VEGF levels was observed as early as 1 week in the 2 cases with higher pretreatment serum VEGF levels. In a single case receiving a strict angio-sonographic evaluation with angio-sonography at 1, 2, 4, 6, 8 weeks, a reduction in tumor vascularization was observed as early as 2 weeks but standardized tumor response based on CT was reported only after 9 months. A reduction in tumor vascularization observed before a reduction in tumor size coupled with the observation that the perfusion is mainly reduced in the central part of the treated tumors is in line with recently performed studies of monitoring antiangiogenic therapy with vascular functional imaging. Imatinib-mediated antiangiogenic properties have been demonstrated in experimental models and in vivo in CML and neuroblastoma. Imatinib could induce antiangiogenic effects in GIST. This effect could be easily monitored with angio-sonography. Large studies are warranted.

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A phase I study of AEE788, a novel multi-targeted inhibitor of ErbB and VEGF receptor family tyrosine kinases

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Background: Combined blockade of multiple signal transduction pathways may result in improved antitumor effects. AEE788 is an orally active, reversible, small molecule multi-targeted kinase inhibitor with potent inhibitory activity against ErbB and VEGF receptor family of tyrosine kinases. In preclinical *in vitro* and *in vivo* studies, inhibition of both the ErbB and VEGF receptor pathways has been shown. AEE788 has an IC_{50} of less than 100 nM against EGFR, ErbB2, VEGFR2. This phase I study was to assess the safety, pharmacokinetics (PK), MTD/DLT dose levels, and optimal biological dose of AEE788.
Methods: Patients (pts) with advanced solid tumors were enrolled. Dose escalation approximated a modified Fibonacci series with 3–6 pts/cohort. Safety monitoring included additional cardiac assessment. No prior EGFR/ErbB2 or VEGF/VEGFR directed therapies were permitted. Pharmacodynamic markers were analyzed in pre- and post-treatment skin and tumor biopsies. A 24 hr PK profile was obtained on days 1, 15 and 28, with trough sampling on days 8 and 22.
Results: To date, 27 advanced cancer pts (15 male, 12 female), median age 55 (range 25–78), have been treated with AEE788 at doses of 25 (5), 50 (6), 100 (5), 150 (5) or 225 mg (6) per day. AEE788 was given on a continuous daily schedule. Tumor types treated were breast (5), colon (5), bladder (2), melanoma (2), liver (2), soft tissue sarcoma (2), and 9 other tumor types (1 each). No dose limiting toxicities have been reported. The most common adverse events (>10%) included diarrhea (41%), nausea (33%), fatigue (30%), skin rash (18%) anorexia (15%), cough (15%), vomiting (15%), anemia (11%), asthenia (11%), cancer pain (11%), constipation (11%), pruritus (11%) and pyrexia (11%). 6 pts had diarrhea suspected to be related to AEE788; (50 mg-1 pt grade (gr) 1; 150 mg-1 pt gr 1, 1 pt gr 2; and 225 mg-3 pts gr 1). 5 pts had drug-related skin rash (100 mg-1pt gr 1, 150 mg-1pt gr 1, 225 mg-2 pts gr 1, 1 pt gr 2). There were no study drug-related grade 3 or 4 adverse events or lab abnormalities. There was no QTc > 500 ms in over 1000 ECGs. Exposure to AEE788 increased overproportionately with dose, with estimated half-life of 24–30 hrs. The ratio of an active metabolite (AQM674) to parent (AEE788) was on average ~ 0.7 (range 0.2 to 2). Exposure to AEE788 and AQM674 was similar after 15 and 28 days of dosing (with the exception of the 25 mg dose), suggesting that PK equilibrium was reached on or before day 15. The best response was stable disease (SD). To date, 7 patients have received AEE788 for > 2 cycles. The median number of cycles of AEE788 was 1.6 (range 0.5–8.8).
Conclusion: AEE788 was well tolerated at daily doses up to 225 mg/day. The study is continuing, the MTD/DLT dose level has not yet been reached.

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The abrogation of rapamycin-induced AKT activity by the small molecule IGF-IR inhibitor, AEW541, and the enhanced antitumor activity of combined mTOR and IGF-IR inhibition

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mTOR (the mammalian target of rapamycin) is a serine/threonine kinase that senses nutrient availability and acts as a central regulator of cell growth. mTOR activates p70 S6 kinase (RSK) which increases translation of mRNA with 5′ polypyrimidine tracts and inhibits the translational repressor 4E-BP1. Small molecule inhibitors of mTOR (rapamycin, CCI-779, RAD001) have antitumor activity in pre-clinical cancer models and modest single agent activity in cancer patients. We hypothesized that resistance to mTOR inhibitors may be the result of adaptive induction of parallel survival pathways following mTOR inhibition. We observed that exposure of MDA-468 (breast) and DU-145 (prostate) cells to rapamycin (1nM) resulted in the induction of IRS-1, a key adapter protein in the IGF-1 signal transduction pathway. IRS-1 (insulin receptor substrates-1) mediates insulin and IGF-1 signaling by linking the IGF-1 and insulin receptor tyrosine kinases to multiple downstream signaling proteins, including p85, the regulatory subunit of PI3K, via interaction with the p85 SH2 domain. We found that rapamycin-stimulated IRS-1 induction was accompanied by increased AKT activity and phosphorylation of its downstream substrate GSK3β. Furthermore, inhibition of IGF-1R with AEW541, an inhibitor of IGF-1 receptor tyrosine kinase, abrogated the upregulation of p-Akt seen following mTOR inhibition. This combination of rapamycin and AEW541 also synergistically inhibited cell growth. The data suggest that IGF-1R inhibition sensitizes cancer cells to mTOR inhibition by counteracting the rapamycin-induced positive feedback upregulation of IGF-1 signalling molecules. This evidence provides a rationale for testing combined inhibition of IGF-1R and mTOR in cancer patients.

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CF101, an agonist to the A3 adenosine receptor enhances the chemotherapeutic effect of 5-fluorouracil in a colon carcinoma murine model

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Background: The molecular mechanism underlying chemo-resistance of tumor cells to cytotoxic drugs entails high levels of NF-kB and the upstream kinase PKB/Akt, acting as inhibitors of apoptosis. A3 adenosine receptor (A3AR) activation with the specific agonist CF101 has been shown to inhibit the development of colon carcinoma growth in vitro and in vivo. In addition CF101 protected mice against myelotoxic effects of chemotherapy via its capability to induce G-CSF production. In this study we examined the